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ACCELERATED COMMUNICATION

Factors Determining the Activity of 2′,3′-Dideoxynucleosides in Suppressing Human Immunodeficiency Virus *In Vitro*

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SUMMARY

Mitsuya and Broder [*Proc. Natl. Acad. Sci. USA* **83**:1911–1915 (1986)] demonstrated that every purine (adenosine, guanosine, and inosine) and pyrimidine (cytidine and thymidine) nucleoside containing the 2′,3′-dideoxyribose configuration, when evaluated against human immunodeficiency virus (HIV) *in vitro*, significantly suppressed both the infectivity and the cytopathic effect of the virus, with 2′,3′-dideoxycytidine (ddCyd) being the most potent of the series (total antiviral protection at 0.5–1.0 μM). We have compared three factors likely to be of significance in determining the pharmacological activity of these compounds, i.e., (i) their abilities to influence pool sizes of physiological deoxynucleoside-5′-triphosphates, (ii) their capacity to generate the corresponding 2′,3′-dideoxynucleoside-5′-triphosphates, and (iii) the effectiveness of these nucleoside-5′-triphosphates as inhibitors of HIV reverse transcriptase. In MOLT-4 cells (a human T cell line), ddCyd was the compound most efficiently converted to its 5′-triphosphate, whereas 2′,3′-dideoxyguanosine and 2′,3′-dideoxythymidine were the compounds least efficiently converted, generating levels of their corresponding 5′-triphosphates less than 0.1% of that seen with ddCyd when these nucleosides were compared on an equimolar basis (5 μM). The 3′-azido analogue of 2′,3′-dideoxythymidine fell intermediate between these two extremes. As inhibitors of HIV reverse transcriptase, however, all the 5′-triphosphates, with the exception of 2′,3′-dideoxyinosine-5′-triphosphate, fell within a narrow range of activity (K_i, 0.10–0.26 μM), affinities some 40–60 fold greater than those of the corresponding physiological 2′-deoxynucleoside-5′-triphosphates. Significant alterations in pool sizes of physiological 2′-deoxynucleoside-5′-triphosphates were not observed at pharmacologically effective drug levels. The relative ability of 2′,3′-dideoxynucleosides to generate 5′-triphosphates intracellularly thus correlates much more closely than do the other two factors examined, in capacity to block HIV replication. These studies support the conclusion that, for purposes of design of new compounds of this general class, factors influencing efficiency of nucleotide formation and degradation (e.g., membrane transport mechanisms, affinities for nucleoside kinases and for nucleotide kinases and phosphatases) may be of equal or even greater importance than differences in the relative abilities of the resultant 2′,3′-dideoxynucleoside-5′-triphosphates to inhibit the viral reverse transcriptase.

The HIV has been recognized as the etiological agent of acquired immunodeficiency syndrome (1, 2). A number of 2′,3′-dideoxynucleosides and related compounds inhibit the *in vitro* infectivity and cytopathic effect of the HIV retrovirus (3, 4); of this number, two, AZT and ddCyd, have shown anti-HIV activity in human subjects either alone or in alternate combination therapy (5, 6). Other members of this general class of compounds (ddAdo and ddIno) are presently in phase I clinical trial.

It is generally assumed that these compounds have a common mechanism of action, i.e., they are phosphorylated by nucleoside and nucleotide kinases to their respective 5′-triphosphates (7–10) and, as the latter, act to inhibit retroviral reverse transcriptase (7, 11), thus slowing the incorporation of physiological 2′-deoxyribonucleotides into viral DNA. In addition, these compounds can themselves serve as alternate substrates for the viral DNA polymerase and become incorporated into the growing DNA chain, thus elongating the chain by one residue and terminating further DNA synthesis (12).

ABBREVIATIONS: HIV, human immunodeficiency virus; AMV, avian myeloblastosis virus; ddCyd, 2′,3′-dideoxycytidine; ddThd, 2′,3′-dideoxythymidine; ddGuo, 2′,3′-dideoxyguanosine; ddAdo, 2′,3′-dideoxyadenosine; ddIno, 2′,3′-dideoxyinosine; dTTP, 2′-deoxythymidine-5′-triphosphate; dCTP, 2′-deoxycytidine-5′-triphosphate; dGTP, 2′-deoxyguanosine-5′-triphosphate; dATP, 2′-deoxyadenosine-5′-triphosphate; AZT, 3′-azido-2′,3′-dideoxythymidine-5′-triphosphate; ddCTP, ddCyd-5′-triphosphate; ddITP, ddIno-5′-triphosphate; ddTTP, ddThd-5′-triphosphate; ddGTP, ddGuo-5′-triphosphate; ddATP, ddAdo-5′-triphosphate; IL-2, interleukin-2; RT, reverse transcriptase; HPLC, high performance liquid chromatography; AZTTP, AZT-5′-triphosphate.

The 2′,3′-dideoxynucleosides and related compounds differ by several orders of magnitude in their effectiveness as inhibitors of HIV replication *in vitro*, e.g., in the ATH8 test system the IC_{50} ranges from approximately 0.1 μM for ddCyd to 150 μM for ddThd under conditions of high viral load (4). Similar wide variations are seen in other test systems. Despite its potential significance for drug design, no consistent explanation has yet emerged for the wide variation in antiretroviral activity within this series. The most likely explanations for these differences, however, would appear to be three in number, i.e., differences in the ability of the 2′,3′-dideoxynucleosides to perturb endogenous pools of physiological deoxynucleosides, differences in the relative ability of the dideoxynucleosides to generate their corresponding 5′-triphosphates, and differences in the relative ability of the latter compounds to act as alternate substrate inhibitors for the enzyme. We have therefore carried out a comparison of these properties for the four prototype dideoxynucleoside analogues ddCyd, ddThd, ddAdo and ddGuo, with supplementary studies also carried out with the closely related compounds ddIno and AZT. A preliminary account of some of these studies has appeared (13).

Experimental Procedures

Materials

Isotopes. [^{3}H]dTTP, [^{3}H]dCTP, [^{3}H]dGTP, [^{3}H]dATP, and [^{3}H] poly(A) used in these experiments were purchased from ICN Corp. (Irvine, CA), and [5,6-^{3}H]ddCyd (10 Ci/mmol), [*methyl*-^{3}H]ddThd (10 Ci/mmol), [2′,3′-^{3}H]ddAdo (30 Ci/mmol), and [8-^{3}H]ddGuo (3 Ci/mol) were obtained from Moravek Biochemicals (Brea, CA). [2′,3′-^{3}H]ddIno (30 Ci/mol) was made by enzymatic deamination of ddAdo, utilizing calf intestinal adenosine deaminase (Sigma, St. Louis, MO) as previously described (14).

Chemicals. 2′,3′-ddTTP, ddGTP, ddATP, ddITP, ddCTP, poly(rA)·$(dt)_{15}$ poly(rC)·$dG)_{12-18}$, poly(dA-T), poly(I), oligo$(dC)_{18}$, and poly(T) were purchased from Pharmacia (Piscataway, NJ). AZTTP was generously supplied by Drs. J. Balzarini and E. DeClercq, Rega Institute (Leuven, Belgium). dCTP, dATP, dGTP, and dTTP were purchased from Sigma. Dithiothreitol was the product of Boehringer Mannheim Biochemicals (Indianapolis, IN).

Enzymes. AMV RT was purchased from Pharmacia and affinity-purified HIV RT from HIV-infected human T cells (15) was provided by the Department of Cell Biology, Bionetics Research, Inc. (Rockville, MD). One unit is the enzyme activity that incorporates 1.0 nmol of [^{3}H]deoxythymidine-5′-monophosphate into acid-insoluble products in 10 min at 37° with poly(rA)·$(dT)_{15}$ as template-primer. Whatman 3 MM paper, used for the reverse transcriptase assays, was purchased from Whatman, Inc., Clifton, NJ.

Cells. MOLT-4, CEM, and H9 cells (the latter from Dr. M. Popovic of the National Cancer Institute), all verified to be free of mycoplasma, were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (56°, 30 min), 50 units/ml of penicillin, 50 μg/ml of streptomycin, and 4 mM L-glutamine at 37° in a humidified atmosphere of 95% air/5% CO_2. Cells were verified to be in logarithmic growth at the time of use. ATH8 cells (mycoplasma-free) were grown by previously described methods (3). Human lymphocytes were obtained by elutriation and cultured at an initial cell concentration of 4 × 10^5 cells/ml in the presence of phytohemagglutinin, 1:200. After 5 days in culture, IL-2 was added to the medium (20 units of recombinant IL-2/ml plus 15% of human purified IL-2). Lymphocytes were cultured for an additional 7 days, reaching a cell concentration of 2.1 × 10^8 cells. The lymphocytes were then split into individual flasks containing 60 × 10^6 cells/flask in 75 ml of the same IL-2-containing medium. Incubations with 2′,3′-dideoxynucleosides were then carried out as with the other cell lines, except that cells were harvested for 2′-deoxynucleoside-5′-triphosphate analysis at both 24 and 48 hr.

Methods

Formation of 5′-triphosphates from 2′,3′-dideoxynucleosides. Logarithmically growing MOLT-4 cells were suspended in RPMI 1640 medium plus 10% fetal calf serum that had been heat treated (56°, 24 hr) to inactivate adenosine deaminase (16). After 24-hr incubation at 37°, cell extracts were prepared and subjected to ion exchange (Partisil-10 SAX) HPLC chromatography for determination of 5′-triphosphate levels as previously described (8, 10, 14). In all cases, the elution time of radioactive peaks was determined by comparison with authentic standards.

Preparation of poly(rI)·$(dC)_{18}$. Poly(rI)·$(dC)_{18}$ was made by annealing the respective 18-base oligomer to the ribopolymer chain. One milligram of poly(I) (20 units) was mixed with 5 units of oligo$(dC)_{18}$ in the presence of 100 mM Tris, pH 8.2, and 1 mM $MgCl_2$. The mixture was incubated at 56° for 15 min, then cooled on ice immediately and diluted 4 times by the addition of 0.1 mM Tris-HCl, pH 8.2. It was stored at −20° until use.

Reverse transcriptase activity assay. All reactions were carried out in a total volume of 25 μl. The final concentrations in the reaction assay were 0.1 M Tris·HCl, pH 8.2, 1 mM dithiothreitol, and 5 μg of bovine serum albumin. In different template-primer-substrate systems, $MgCl_2$, [^{3}H]dATP, [^{3}H]dCTP, [^{3}H]dGTP, [^{3}H]dTTP, and reverse transcriptases of AMV or HIV were added, as indicated in the legends for figures and tables. With ddATP as inhibitor, the template was poly(dA-T); with ddCTP, poly(rI)·$(dC)_{18}$; with ddGTP and ddITP, poly(rC)·$(dG)_{12-18}$; and with ddTTP, poly(rA)·$(dT)_{15}$. Under the conditions indicated, after a 60-min incubation at 37°, the reaction was terminated by heating at 95° for 1 min. Reaction products were separated from ^{3}H-labeled precursors by ascending 3 MM paper chromatography in a solvent system that contained 55% ethanol/20% formic acid/25% H_2O (v/v/v) for 16 hr. The locations of polymerized products were determined by UV illumination; in all cases they remained as sharp spots at the origin. Spots containing such products were cut out, eluted with 1 ml of distilled water, and counted in Beckman scintillation fluid.

Determination of deoxynucleoside-5′-triphosphate pool sizes. Deoxynucleoside triphosphate pools were analyzed using the method of Garrett and Santi (17) with the following modifications. The trichloroacetic acid extraction method of Khym (18) was used instead of the perchloric acid method. Cyclohexyl ammonium chloride, 5 M, and 2 M glycerol were substituted for the methylamine and rhamnose, respectively, as suggested by Dr. W. Plunkett, University of Texas M. D. Anderson Hospital and Tumor Institute.[1] The chromatographic system used a Whatman Partisphere 5 SAX 12.5 × 0.4 cm column at 45° with gradient elution from 0.02 M ammonium phosphate, pH 3.5 to 0.7 M ammonium phosphate, pH 3.5, over 40 min at 2 ml/min. Detection was by UV absorption at 254 nm.

Results

Formation of 5′-triphosphates from 2′,3′-dideoxynucleosides. When these compounds were compared at equimolar levels (5 μM), wide variation was observed in the ability of MOLT-4 cells to generate 5′-triphosphates from 2′,3′-dideoxynucleosides; ddCyd was more than 1500-fold more effective as a precursor of its corresponding 5′-triphosphate than was the least active member of the series, ddThd (Table 1). AZT was intermediate between these two extremes, i.e., some 35-fold more effective than ddThd and 40-fold less effective than

[1] Personal communication.

TABLE 1

Intracellular levels of 5′-triphosphates generated from 2′,3′-dideoxynucleosides

Molt-4 cells were suspended in RPMI 1640 medium plus 10% fetal calf serum that had been heat-treated (56°, 24 hr) to inactivate adenosine deaminase. Initial cell density, 3×10^5 cells/ml. Cells were exposed to ^{3}H-labeled dideoxynucleosides (specific activity, 1 μCi/nmol) at a concentration of 5 μM for 24 hr before harvesting. In the case of purine nucleoside precursors, nucleotides were extracted from the pelleted cells with 60% methanol and the extracts were immediately heated at 95° for 1 min to inactivate phosphatase activity. After centrifugation at 12000 × *g*, the methanolic supernatants were analyzed by ion exchange HPLC (10). In the case of pyrimidine nucleoside precursors, the pelleted cells were extracted with 10% trichloroacetic acid and the extracts were neutralized with tri-*n*-octylamine in Freon before HPLC analysis (8). Activities are expressed as intracellular concentration (μM) of 2′,3′-dideoxynucleoside-5′-triphosphate (mean ± standard error), assuming a mean cellular volume of 1 pl.

Dideoxynucleoside	2′,3′-Dideoxynucleoside-5′-triphosphate
	μM
ddAdo	0.085 ± 0.016
ddGuo	0.046 ± 0.002
ddIno	0.064 ± 0.003[a]
ddThd	0.038 ± 0.004
ddCyd	58.6 ± 15.0[b]
AZT	1.36 ± 0.80

[a] Concentration of ddATP formed from ddIno.

[b] No deamination of ddCyd to ddUrd was noted under the conditions of these experiments; with mammalian kidney cytidine deaminase, an extremely slow conversion of ddCyd to ddUrd can be demonstrated, but the reaction rate is approximately 0.1% of that seen with the natural substrate cytidine (19).

ddCyd in generating its corresponding 5′-triphosphate. No interconversion of nucleosides was noted in these experiments except for the deamination of ddAdo to ddIno previously described (10).

Inhibition of HIV RT and AMV RT by dideoxynucleoside-5′-triphosphates. Six 2′,3′-dideoxynucleoside-5′-triphosphates were then tested for their inhibitory potency against HIV RT and AMV RT in four template-primer systems. Previous studies showed that all the parent dideoxynucleosides, ddAdo, ddCyd, ddGuo, ddIno, ddThd, and AZT, inhibited the replication of HIV in the ATH8 test system (3, 4). Our results show that the 5′-triphosphates of these six dideoxyribonucleosides inhibit HIV and AMV RT DNA polymerase activities at submicromolar concentrations (Table 2). All the 2′,3′-dideoxynucleotides yielded formally competitive inhibition with respect to their 2′-deoxy counterparts under the conditions used in these studies; typical reciprocal plots for dCTP:ddCTP are shown in Fig. 1. The kinetic parameters are summarized in Table 2; ddGTP, ddCTP, ddATP, AZTTP, and ddTTP are comparably strong inhibitors of both AMV and HIV RT with K_i values ranging from 0.10 to 0.26 μM under these reaction conditions; ddITP, which is a mismatching nucleotide analog of dGTP, was a much weaker inhibitor than the other dideoxynucleotides examined but was a stronger inhibitor of HIV RT than of AMV RT.

Effect of 2′,3′-dideoxynucleosides on pool sizes of deoxynucleoside-5′-triphosphates. The effect of 2′,3′-dideoxynucleosides on intracellular levels of the physiological 2′-deoxynucleoside-5′-triphosphates dCTP, dTTP, dATP, and dGTP was next examined in both MOLT-4, H9 CEM and ATH8 cells, and phytohem agglutinin-stimulated human lymphoblasts. Drug concentration ranges used were those over which the individual dideoxynucleosides exhibited their antiretroviral effects. No consistent changes were observed in endogenous deoxynucleoside triphosphate levels except for an increase in dCTP after exposure to high levels of AZT (50 μM) in H9, MOLT-4, CEM, and ATH8 cells (Tables 3 and 4 and data not shown). In these four cell lines, AZT exposure resulted in no detectable decrease in dTTP levels; in phytohemagglutinin-stimulated human lymphoblasts; however, a slight but significant decrease (30–40%) was noted in dTTP pool sizes after both 24 hr and 48 hr exposure to 50 μM AZT. 5-Fluoro-2′-deoxyuridine was utilized as a positive control; exposure of H9 cells to the latter agent at a concentration of 2 μM resulted in a decrease in dTTP levels to 11% of control values by 24 hr (Table 4).

Discussion

The dideoxynucleosides vary widely in their ability to inhibit the cytopathic effect and infectivity of human immunodeficiency virus. Mitsuya and Broder (4) found, for example, that ddCyd afforded 50% protection against the viral cytopathic effect at 0.1 μM (even using a high multiplicity of infection), whereas approximately 150 μM ddThd was required for comparable protection, a 1500-fold difference in potency between these two compounds. Other dideoxynucleosides fall intermediate between these two extremes.

When these antiviral effects are correlated with the three factors examined here, close correspondence was seen only with efficiency of 5′-triphosphate formation, with ability of 2′,3′-dideoxynucleosides to generate nucleotides varying over more than a 1000-fold range. ddCyd generated the greatest level of intracellular 5′-triphosphates among the compounds tested and also exhibited the greatest antiviral potency in the ATH8 system whereas ddThd showed the lowest ability to generate 5′-triphosphate and the lowest antiviral activity. No such variation was observed, however, in the relative activities of these

TABLE 2

Inhibition of HIV and AMV reverse transcriptase by 2′,3′-dideoxynucleoside 5′-triphosphates

Assays were performed as described in Materials and Methods. K_m and K_i values were determined from replots of the slopes of reciprocal plots versus 2′,3′-dideoxynucleoside-5′-triphosphate concentrations. Values are the means ± standard error from three separate determinations.

Substrate-inhibitor	Template-primer	AMV RT			HIV RT		
		K_m	K_i	K_m/K_i	K_m	K_i	K_m/K_i
		μM				μM	
dATP-ddATP	Poly(dA-T)	10.0 ± 0.4	0.17 ± 0.09	58.8	11.2 ± 2.9	0.22 ± 0.12	50.9
dCTP-ddCTP	Poly(rI)·$(dC)_{12-18}$	5.7 ± 0.5	0.11 ± 0.06	51.8	6.5 ± 3.1	0.26 ± 0.03	25.0
dGTP-ddGTP	Poly(rC)·$(dG)_{12-18}$	25.1 ± 8.7	0.24 ± 0.013	105	16.5 ± 0.8	0.15 ± 0.08	110
dGTP-ddITP	Poly(rC)·$(dG)_{12-18}$	12.8 ± 2.2	29.9 ± 5.0	0.43	17.4 ± 0.6	2.47 ± 0.82	7.0
dTTP-ddTTP	Poly(rA)·$(dT)_{15}$	7.5 ± 2.9	0.17 ± 0.10	44.1	2.06 ± 0.62	0.19 ± 0.10	10.8
dTTP-AZTTP	Poly(rA)·$(dT)_{15}$	6.9 ± 0.9	0.12 ± 0.04	57.5	1.25 ± 0.06	0.10 ± 0.02	12.5

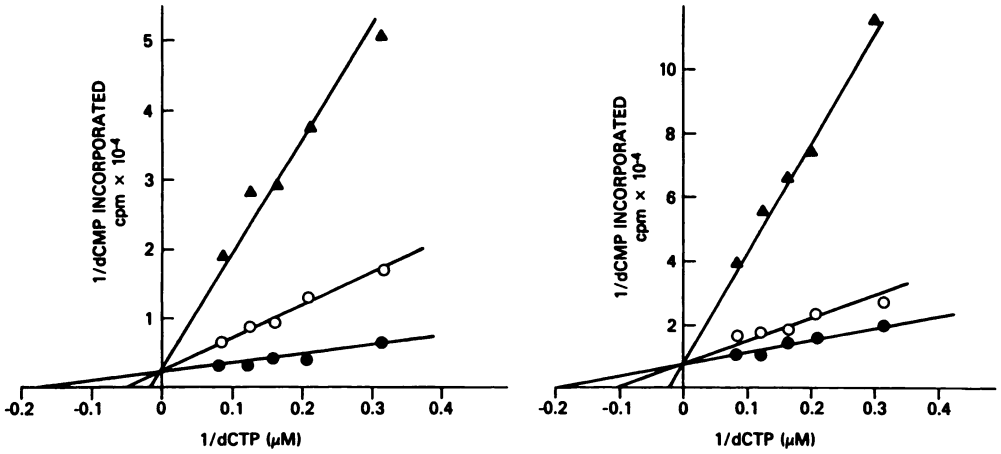

Fig. 1. Inhibition of RT by ddCTP, *Left panel*, AMV RT inhibition by ddCTP. Assays were performed in a final volume of 25 μl, which contained fast protein liquid chromatography-purified AMV RT (2 units), poly(dC)$_{18}$ (1.25 μg), [^{3}H]dCTP (3.125–12.5 μM; specific activity, 24 Ci/mmol), and $MgCl_2$ (5.0 mM). ●, Without ddCTP; ○, with ddCTP (0.25 μM); and ▲, with ddCTP (2.5 μM). *Right panel*, HIV RT inhibition by ddCTP. Assays were performed in a final volume of 25 μl, which contained HIV RT (0.9 units), poly(dC)$_{18}$ (1.25 μg), [^{3}H]dCTP (3.125–12.5 μM; specific activity, 24 Ci/mmol), and $MgCl_2$ (2.5 mM). ●, Without ddCTP; ○, with ddCTP (0.25 μM); ▲, with ddCTP (2.5 μM).

TABLE 3

Intracellular levels of deoxyribonucleoside-5′-triphosphate levels in MOLT-4 cells exposed to 2′,3′-dideoxynucleoside

MOLT-4 cells were exposed to the dideoxynucleoside level indicated for 24 hr. Cells were extracted and 2′-deoxynucleoside-5′-triphosphate levels determined by a modification of the method Garrett and Santi (17). Values shown are the mean of duplicate assays, with the values obtained in different assays varying by less than 10%.

Drug level	dCTP	dTTP	dATP	dGTP
μM	*pmol/10^6 cells*			
0				
ddCyd	9.8	44.0	43.0	19.9
1	9.0	45.6	39.6	17.3
5	11.1	43.0	37.9	17.5
10	13.9	53.2	43.5	19.6
ddAdo				
100	10.3	50.2	72.3	33.2
1000	12.5	46.2	59.0	27.0
AZT				
5	10.4	54.3	53.5	20.7
10	10.7	54.9	46.5	19.0
50	11.9	55.4	41.2	10.7

TABLE 4

Intracellular levels of deoxyribonucleoside-5′-triphosphate levels in H9 cells exposed to 2′,3′-dideoxynucleosides

H9 cells were exposed to the dideoxynucleoside level indicated for 24 hr. Cells were extracted and 2′-deoxynucleoside-5′-triphosphate level determined by a modification of the method of Garrett and Santi (17). Values shown are the mean of duplicate assays, with the values obtained in different ways varying by less than 10%. FUdR, 5′-fluoro-2′-deoxyuridine.

Drug level	dCTP	dTTP	dATP	dGTP
μM	*pmol/10^6 cells*			
0				
ddCyd	7.5	28.9	11.2	2.7
1	9.9	25.6	10.7	2.5
5	8.5	26.7	8.5	3.3
10	10.6	30.6	11.5	3.2
AZT				
5	6.9	27.8	10.1	3.1
10	8.6	27.3	10.2	3.7
50	12.7	33.1	12.9	2.8
FUdR				
2	19.2	3.5	24.6	1.8
5	18.4	3.3	24.9	1.8

compounds as inhibitors of the viral DNA polymerase, with K_i values under our assay conditions ranging from 0.15 to 0.26 μM for the four "physiological" dideoxynucleoside analogues (Ado, Guo, Cyd, and dThd) for the HIV enzyme and from 0.11 to 0.24 μM for the avian myeloblastosis enzyme. The only apparent exception to fall outside this relatively narrow range was ddITP (K_i values of 2.5 and 29.9 for HIV and AMV respectively). We have recently shown, however, that ddITP is not generated intracellularly and that the corresponding 2′,3′-dideoxynucleoside, ddIno, acts instead as a precursor of ddATP (14). Similarly, no correlation was seen between the third factor examined, the ability of the test compounds to modify deoxynucleotide pool sizes, and their antiviral activity; we have been able to confirm the recent observation of Harrington and co-workers (20), that AZT results in an increase in dCTP in human T lymphocytes, but not the earlier observation of Furman *et al.* (7), that this agent causes a significant decrease in both dCTP and dTTP pools. Should these preliminary conclusions be borne out on subsequent studies, the implication in terms of drug design would appear to be that although the ability to inhibit retroviral polymerase is a *sine qua non* for antiretroviral activity, of equal and possibly greater importance, with respect to the design of nucleoside analogues, is the ability of the compounds to generate pharmacologically effective levels of 5′-triphosphate. This value, in turn, is the summation of several processes, among which are the ability of the test compound to enter and leave cells, affinity for the appropriate nucleoside and nucleotide kinases, and susceptibility of the resulting 5′-triphosphate to attack by phosphatases.

Information on these properties for individual members of the present series is incomplete, although a number of isolated observations have been made. AZT is phosphorylated by thymidine kinase and thymidylate kinase successively (7). ddCyd is phosphorylated to 2′,3′-dideoxycytidine monophosphate by deoxycytidine kinase, with a K_m of 180–200 μM (9, 21). ddAdo is activated, although inefficiently, by both deoxycytidine kinase and adenosine kinase (10) but, in addition, undergoes deamination to ddIno and phosphorylation of the latter by still undefined kinases or phosphotransferases (22) to 2′,3′-dideoxyinosine monophosphate, with reconversion to 2′,3′-dideoxyadenosine monophosphate at the monophosphate level (14). ddGuo gives rise to detectable ddGTP (Table 1), but its anabolic route has not yet been defined. Cell entry of ddCyd appears to utilize, at least in part, the pyrimidine nucleoside transport mechanism (8), whereas AZT, and possibly ddAdo, enter cells by passive diffusion (14, 23). The relative susceptibility of the corresponding 5′-triphosphates to phosphatases has not yet been studied, although half-times of disappearance in MOLT-

4 cells of 2.6 hr for ddCTP (9) and of 10–24 hr for ddATP (24) have been determined.

The formally competitive nature of the inhibition of dideoxynucleotides of deoxynucleotide incorporation into template-primer is compatible with strong interaction of the dideoxy analogues with HIV reverse transcriptase, with the affinity of the analogues under our reaction conditions being some 40–60-fold that of the corresponding natural substrates. As discussed above, however, the relative pharmacological activity of the dideoxynucleosides cannot be explained on these grounds because the affinities of their 5′-triphosphates for the enzyme are of the same order of magnitude whereas the antiviral potencies of the parent compounds vary over more than a 1000-fold range.

The dideoxynucleosides, however, differ from other classes of RT inhibitors in also possessing the ability to act as chain terminators. The formally competitive nature of the inhibition observed in the present studies would indicate that, although incorporation into template-primer (i.e., chain termination) may be taking place, it appears on the basis of the kinetics of the inhibition not to be a quantitatively significant process under the reaction conditions used here. That such HIV-transcriptase-catalyzed insertion does take place under somewhat different experimental conditions has been demonstrated for the four dideoxynucleotides ddCTP, ddATP, ddGTP and ddTTP (12). In the latter study, however, little variation was noted in the chain-terminating ability of the dideoxynucleotides, indicating that differences in the efficiency of the chain termination reaction would also appear to be an unlikely explanation for the unusually wide range of variation (more than 3 orders of magnitude) in the antiviral potency of these compounds. We believe that these observations may have useful implications for both the design and pharmacological assessment of other compounds of this same general class.

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